REMARKS

Reconsideration is respectfully requested.

Claims 15, 17, 25 and 26 are amended herein. Claims 16 and 18 are canceled. The remaining claims are not amended.

Withdrawn claims 1-14 are canceled.

Claims 23 and 24 are withdrawn, but in view of the arguments below for the allowability of claim 15, it is respectfully submitted that claims 23 and 24 should be given consideration, since claim 15 was indicated to be generic.

The Office Action includes an objection to the abstract. The Abstract is amended herein with attention to the points noted by the Examiner.

The Office Action objects to the title. In reply, the title is amended. We thank the Examiner for suggesting a title, which has been adopted, with slight modification.

Claims 20, 25, 26 and 27 are objected to as being multiple dependent claims that depend on another multiple dependent claim. Amendments are made herein to claims 25 and 26 to address this issue. With regard to claim 20, it depended only on claims 15 and 19, and neither of claims 15 or 19 is multiple dependent, so it is believed that claim 20 should have been examined on the merits.

Claims 15-19, 21 and 22 rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to

particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner considers that the terms “moist” and “close-packed” are not sufficiently definite. Applicant amends claim 15 herein to address these objections. Regarding “moist”, claim 15 is amended to recite that the atmosphere is 50% or higher humidity, which is supported by the specification at page 22, lines 4 and 5, for example. Regarding “close-packed”, the amendment to claim 15 replaces this term with "packed".

The Examiner objected to “if required” in claim 16, so this is amended to instead be “optionally”, and, is incorporated into claim 15, as claim 16 has been incorporated therein.

In claim 17, the Examiner objects to the “and/or” language, so the amendment replaces this with “or”.

Claims 15-19, 21 and 22 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over JP2001-157574 A combined with Widawski et al (Nature, Vol. 369, 2 June 1994, pages 387-389).

Applicants respectfully traverse.

Applicants obtained a translation of JP2001-157574 (hereinafter, Publication 1) from the JPO website, copy attached for reference.

Publication 1 recites in claim 1 a "honeycomb structure obtained by casting a hydrophobic organic solvent solution of a polymer comprising 50 to 99 w/w% of a biodegradable polymer and

50 to 1 w/w% of an amphipathic polymer on a substrate in the atmosphere having a relative humidity of 50 to 90%, letting said organic solvent evaporate off gradually and at the same time forming dew on the surface of said casting liquid, and evaporating minute droplets resulting from said dew condensation".

At paragraph [0010], Publication 1 describes that for the biodegradable polymer here, for instance, use is preferably made of a biodegradable aliphatic polyester such as polylactic acid, polyhydroxy butyric acid, polycaprolactone, polyethylene adipate and polybutylene adipate; and an aliphatic polycarbonate such as polybutylene carbonate and polyethylene carbonate in view of solubility in organic solvents. Of these, preference is given to polylactic acid, and polycaprolactone because of availability and cost.

However, there is nothing said about using a poly(meth)acrylate as the biodegradable polymer.

Further, at paragraph [0007], Publication 1 describes that not until now is there any honeycomb structure-providing biodegradable material that can maintain its own structure for the time required for cell culture and can break down in a longer time than that. In other words, it is a requirement to use a biodegradable material in applying the honeycomb structure, in combination with cell engineering and cell culture technology, to such medical purposes as artificial organs.

However, polystyrene is in no sense a biodegradable material (polymer); so it would have been excluded from the invention set forth in Publication 1, and more specifically is taught away from by Publication 1.

Further, Widawski, Nature, Vol. 369, 2 June 1994, pp 387-38911 (hereinafter called Publication 2 for short) describes that there is a honeycomb structure obtained in which a porous honeycomb structure comprising polystyrene is peeled off by an adhesive tape thereby obtaining a honeycomb structure having minute projections lined up on the releasing surface.

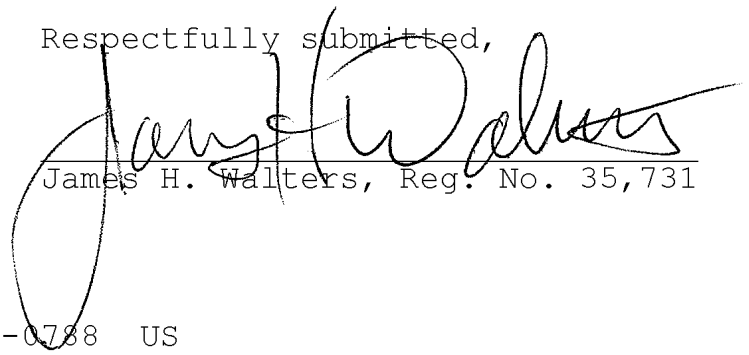
However, since Publication 1 says nothing about the poly(meth)acrylate and polystyrene used as the polymer when the honeycomb structure is obtained in the invention as recited in the claim after amendment, the invention of this application after amendment would not be taught or suggested by Publication 1 or Publication 2, whether considered alone or when combined.

In light of the above noted amendments and remarks, this application is believed in condition for allowance and notice thereof is respectfully solicited. The Examiner is asked to contact applicant's attorney at 503-224-0115 if there are any questions.

It is believed that no further fees are due with this filing or that the required fees are being submitted herewith. However, if additional fees are required to keep the application pending,

please charge deposit account 503036. If fee refund is owed, please refund to deposit account 503036.

Respectfully submitted,



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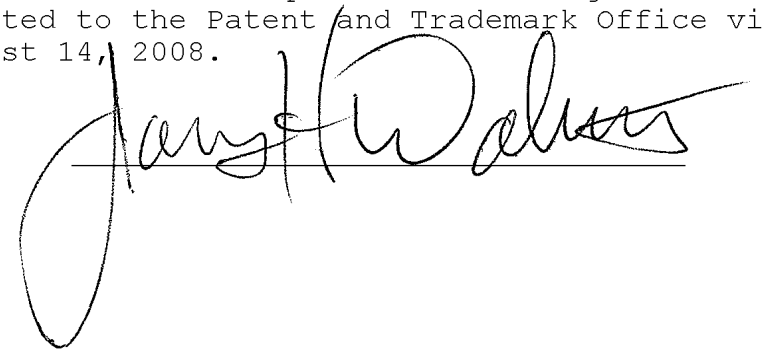

(Publication 1)

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2001-157574

(43)Date of publication of application : 12.06.2001

(51)Int.Cl. C12M 3/00
C08J 9/00
C12N 5/06
// C08L101/16

(21)Application number : 11-340568

(22)Date of filing : 30.11.1999

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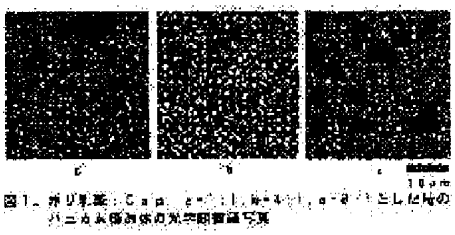
(54) HONEYCOMB STRUCTURE, METHOD FOR PREPARING THE STRUCTURE, FILM AND CELL CULTURE BADE USING THE STRUCTURE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a step when a base and/or a cell forms a three dimensional structure in culturing a cell.

SOLUTION: A hydrophobic organic solvent solution of a polymer comprising 50-99% of a biodegradable polymer (polylactic acid) and 50-1% of amphiphatic polymer (Cap) is cast to a base at 50-95% relative humidity under atmospheric pressure. The organic solvent is gradually transpired and simultaneously condensed on the surface of the cast solution and minute water drops formed by the condensation are evaporated to give a honeycomb structure. The sheet or the cell culture base is obtained by using this honeycomb structure.



CLAIMS

[Claim(s)]

[Claim 1]Biodegradable polymer a hydrophobic organic solvent solution of polymer in which 50-99 w/w% and amphipathic polymer consist of 50-1 w/w%, A honeycomb structured body obtained by carrying out the cast on a substrate under the atmosphere of 50 to 95% of relative humidity, making it dew on this cast liquid surface at the same time it transpires this organic solvent gradually, and evaporating minute waterdrop produced by this dew condensation.

[Claim 2]A film which consists of a honeycomb structured body of claim 1.

[Claim 3]A honeycomb structured body of claim 1 in which said biodegradable polymer is aliphatic polyester.

[Claim 4]A substrate for cell cultures which consists of the honeycomb structured body according to claim 1.

[Claim 5]The substrate for cell cultures according to claim 4 whose diameter of said honeycomb structured body is 0.1-10 micrometers.

[Claim 6]How to prepare a honeycomb structured body by making it dew on this cast liquid surface at the same time it carries out the cast of the hydrophobic organic solvent which carries out 1-50% content of 50-99% and the amphipathic polymer for biodegradable polymer under relative humidity 50-95% of atmosphere on a substrate and transpires this organic solvent gradually.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention is a thing in connection with the foundation of a cell culture and application which serve as the foundation most in a cell technology and system engineering with prosperous research in recent years by medical science or an agricultural field.That is, a scaffold in case the substrate which is in charge of culturing a cell, and/or a cell form a three-dimensional organism is provided. [0002]
[Description of the Prior Art]In the interaction of a cell and material, it is known that a cell will be influenced not only with the chemical character of a material-list side but with detailed shape.Then, when aiming at the function control of a cell from viewpoints of system engineering etc., processing of the chemical nature of the material-list side in contact with a cell and the both sides of a detailed structure becomes important.The size control of the cell adhesion side using the micro pattern art used for semiconductor industry etc. as surface treatment art as a surface micro-processing method, Introduction of the minute groove structure to a substratum and production of the detailed unevenness by a microsphere are performed, and it is known that the surface fine structure will affect growth of a cell, etc. greatly. [0003]The actual condition is having many problems, such as very advanced art's being required for surface setting out using such micro pattern art, and mass production being possible, and becoming a high cost which is not.it is known that the film which has the honeycomb structure of mum scale by carrying out the cast of the diluted solution of the polymer which has a structure completely special as another surface patterning art under high humidity will be obtained. It is the feature that this method is excellent in the economical efficiency which is in charge of patterning. [0004]
Specifically in a science and 1999 using polyphenyl quinolin-block polystyrene which is rod KOIRUJI block polymer which becomes 283 volumes and the page 373 from a hydrophilic block and a hydrophobic block ****, the jib which will be 369 volumes and the page 387 from polystyrene and poly para-phenylene which is upright blocks in Nature and 1994 -- the example using lock polymer is indicated.Thus, at the Prior art, special polymer having a portion with strong autoagglutination power and the portion which reveals pliability was used, these polymer was dissolved in the hydrophobic organic solvent, and the honeycomb structured body was prepared by carrying out the cast of this.On the other hand, this invention persons are SHINSO lids. Films 1998 327 to 329 volumes, Page 854, Supra molecular science 1998 The 5th volume, the page 331 -- and molecular Crystal Liquid Crystal In 1998. The 322nd volume Acrylamide polymer of hydrophilic nature is made the page 305 with a principal chain skeleton, The amphipathic polymer which has a lactose group or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or it has reported giving the thin film which has

honeycomb structure in a way with a similar ion complex of anionic polysaccharides, such as heparin and dextran sulfate, and the long chain alkyl ammonium salt of the 4th class. [0005]However, in these polymer, since it has a fault of it being inferior to the self-independence nature of the honeycomb structured body obtained, or honeycomb structure collapsing temporally, function sufficient as a substrate for cell cultures is not provided. [0006]

[Problem(s) to be Solved by the Invention]When performing a cell culture in a cell technology, system engineering, etc., the substrate used as the scaffold of a cell is required, and it is known like the above-mentioned that a cell will be influenced in the interaction of a cell and material not only with the chemical character on the surface of best but with detailed shape.When aiming at the function control of a cell, a design of the chemical nature of a material-list side and the both sides of structure with a detailed cell in contact with a cell becomes important.With the porous film which has honeycomb structure, a honeycomb pattern provides a cell adhesion side and it is shown that porous structure serves as access to the supporting board of a cell and a supply route of a nutrition. [0007]If a cell is systematized based on this honeycomb structure film, an artificial organ can be considered as that one utilizing method.However, as for this substrate, since embedding inside of the body becomes indispensable when an artificial organ etc. are used, being absorbed to the living body is desirable in the long run.The time which a cell culture takes with the material which gives old honeycomb structure maintains structure stably, and there is nothing that was made from biodegradable material which is disassembled at more than it.In other words, in combining a honeycomb structured body, a cell technology, and cell culture technology, and developing to medical-application ways, such as an artificial organ, it is indispensable to use biodegradable material. [0008]

[Means for Solving the Problem]As a result of inquiring wholeheartedly in consideration of an above-mentioned technical problem and a problem, this invention person is combining a biodegradable polymer and amphipathic polymer at a suitable rate, economical preparation is possible for him, there is independence nature, and it found out giving a also structurally stable honeycomb structured body.That is, this invention is attained by the following. [0009](1) Biodegradable polymer a hydrophobic organic solvent solution of polymer in which 50-99 w/w% and amphipathic polymer consist of 50-1 w/w%, A honeycomb structured body obtained by carrying out the cast on a substrate under the atmosphere of 50 to 95% of relative humidity, making it dew on this cast liquid surface at the same time it transpires this organic solvent gradually, and evaporating minute waterdrop produced by this dew condensation.

A film which consists of a honeycomb structured body of (2) and (1).

(3) A honeycomb structured body of (3) said whose biodegradable polymer is aliphatic polyester.

A substrate for cell cultures which consists of a honeycomb structured body of (4) and (1).

(5) The substrate for cell cultures according to claim 4 whose diameter of said honeycomb structured body is 0.1-10 micrometers.

(6) How to prepare a honeycomb structured body by making it dew on this cast liquid surface at the same time it carries out the cast of the hydrophobic organic solvent which carries out 1-50% content of 50-99% and the amphipathic polymer for biodegradable polymer under relative humidity 50-95% of atmosphere on a substrate and transpires this organic solvent gradually.

[0010]

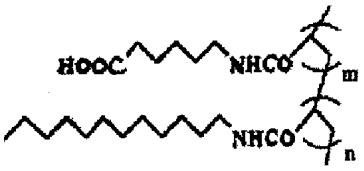
[Embodiment of the Invention]As biodegradable polymer in this invention, polylactic acid and polyhydroxy butanoic acid, Aliphatic series polycarbonate, such as biodegradable aliphatic polyester, such as a

polycaprolactone, polyethylene adipate, and a polybutylene horse mackerel peat, polybutylene carbonate, and polyethylene carbonate, etc. are preferred from a soluble viewpoint to an organic solvent.Especially, polylactic acid and a polycaprolactone are desirable from viewpoints of the ease of acquisition, a price, etc. [0011]From it being indispensable that there is no toxicity if it takes into consideration to use as a cell culture base material as amphipathic polymer used for this invention.A polyethylene glycol / polypropylene-glycol block copolymer, The amphipathic polymer which makes acrylamide polymer a principal chain skeleton and has a lactose group or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or it is desirable to use the amphipathic polymer etc. which made the hydrophilic radical water soluble protein, such as an ion complex of anionic polymers, such as heparin, dextran sulfate, and nucleic acid of DNA or RNA, and long chain alkyl ammonium salt, gelatin, collagen, and albumin. [0012]In producing the honeycomb structured body of this invention, it is required to be nonaqueous solubility as an organic solvent used from it being indispensable to make a minute waterdrop particle form on a polymer solution.As these examples, nonaqueous solubility ketone, such as ester species, such as aromatic hydrocarbon, such as halogen system organic solvents, such as chloroform and a methylene chloride, benzene, toluene, and xylene, ethyl acetate, and butyl acetate, and methyl isobutyl ketone, carbon bisulfide, etc. are mentioned.Whether it uses it alone or uses these organic solvents as a mixed solvent which combined these solvents, they are not cared about.the polymer concentration which amphipathic polymer both persons combine with the biodegradable polymer which dissolves in these -- 0.01 to 10wt% -- it is 0.05 to 5wt% more preferably.The dynamics intensity of the film which will be obtained if polymer concentration is lower than 0.01wt% runs short, and it is not desirable.Honeycomb structure sufficient at more than 10wt% in which polymer concentration becomes high too much is not acquired.The composition ratio of biodegradable polymer and amphipathic polymer is 99:1 to 50:50 (wt/wt).the honeycomb structure in which an amphipathic polymer ratio is uniform at one or less is not acquired -- moreover -- this -- it is not desirable in order that a ratio may be missing from the stability, especially dynamic stability, of the honeycomb structured body obtained or more by 50. [0013]Although the cast of this polymer organic solvent solution is carried out on a substrate in this invention and a honeycomb structured body is prepared, As this substrate, the fluids excellent in products made from an organic solvent-proof, such as inorganic materials, such as glass, metal, and a silicon wafer, polypropylene, polyethylene, and polyether ketone, such as polymers, water, a liquid paraffin, and liquefied polyether, can be used.Especially, when water is used for a substrate, this structure can be independently taken out from a substrate easily by employing efficiently the independence nature which is the feature of this honeycomb structured body, and it is suitable. [0014]The mechanism in which honeycomb structure is formed by this invention is considered as follows.When a hydrophobic organic solvent evaporates, in order to take latent heat, the temperature of the cast philharmonic surface falls, and the drop of minute water condenses and adheres to the polymer solution surface.by work of the hydrophilic portion in a polymer solution, the surface tension between water and a hydrophobic organic solvent decreases, and for this reason, water particles condense and it becomes one lump -- trying -- the time -- carrying out -- stabilizing -- having .A solvent follows on evaporating and it stands in a line in the form in which the drop which carried out the hexagonal form carried out the closest packing, and finally, water flies and polymer remains as a form regularly located in a line with honeycomb shape.Therefore, it is desirable for relative humidity to be in 50 to 95% of range as environment where this film is prepared. At 50% or less, dew condensation of a up to [a cast film] becomes insufficient, and environmental control is not difficultly preferred at not less than 95%.Thus, the size of each of the made honeycomb structured bodies (each) is 0.1 to 10 micrometers, and if it is a size of this range, it can be conveniently used

as a substrate for cell cultures.

[0015]Hereafter, although this invention is explained in detail using an example, this invention is not limited at all by this. [0016]

[Example](Example 1-3) The chloroform fluid (1.0 g/L) of Polly L-lactic acid (molecular weights 85000-160000) and the benzene solution (1.0 g/L) of Cap of formula (I) are mixed at a rate of 1:1, 4:1, and 8:1, The cast was carried out on the glass substrate, it settled under the condition of a room temperature and 80% of humidity, and the honeycomb structured body was prepared by flying a solvent gradually.In this way, the optical microscope photograph of the obtained structure is shown in drawing 1. These films can be picked up with tweezers and it was checked that free-standing is shown. [Formula 1]



Ｃａｐの構造式

[0017](Example 4) Milli-Q water (40 ml) is put into a petri dish (9.3 cm in inside diameter), The chloroform fluid (1.0 g/L) of Polly L-lactic acid (molecular weights 85000-160000) and the benzene solution (1.0 g/L) of Cap which is amphipathic polymer were mixed at a rate of 8:1 (wt%), the 20microl was developed on the water surface, and the collapse film was produced. Then, this polymer solution of 10 moremicrol was dropped, the drop was made to form, and the honeycomb structured body was produced by applying the air of 80% of relative humidity at the speed of 90 ml/min to it. This structure could be dipped up on the frame (phi= 5 mm), and has checked free-standing. [0018](Comparative example 1) Only with the chloroform fluid (1.0 g/L) of Polly L-lactic acid (molecular weights 85000-160000), adjustment of honeycomb structure was tried by the same operation as Example 1.Although the result was shown in drawing 2, the morphology of the obtained film was heterogeneous.

[0019](Comparative example 2) Preparation of the honeycomb structured body was tried on Example 1 and simultaneous conditions only using the Cap solution.The film was beaten by this example while minute waterdrop evaporated, and by it, it did not have free-standing. [0020](Example 1 of an examination) It installed on the glass plate which carried out the poly HEMA coat of the honeycomb film obtained in Example 1, and the cow main artery origin vascular endothelial cell (ECs) was cultured on this.Culture was performed using the IMDM culture medium within CO_2 incubator (CO_2 concentration =5%, temperature =37 **, relative humidity = 80%).Seeding of the ECs was directly carried out on the glass plate of a pHEMA coat as a comparative example, and it cultivated on the same conditions.In the former, it turned out that it pasted up well, the cell is extended and the film of a honeycomb structured body is functioning as a scaffold of a cell.On the other hand, in the latter, ECs was not pasted up at all. [0021]

[Effect]As mentioned above, according to the method of this invention, preparation of the honeycomb structured body which used biodegradable polymer as the main ingredients and which was arranged regularly is attained simple, and offer of the film and cell culture base material using this is attained.

[Translation done.]

DRAWINGS

[Drawing 1]

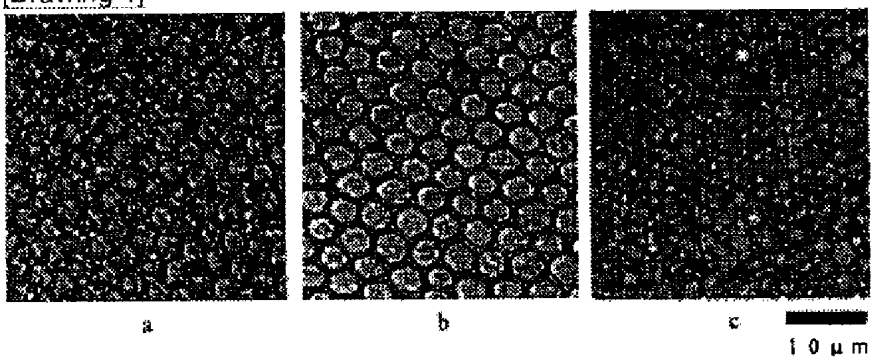


図１．ポリ乳酸：Ｃａｐ　ａ＝１：１，ｂ＝４：１，ｃ＝８：１とした時のハニカム構造体の光学顕微鏡写真

[Translation done.]

WRITTEN AMENDMENT

---------- [Written amendment]
[Filing date]January 14, Heisei 12 (2000.1.14)
[Amendment 1]
[Document to be Amended]Specification
[Item(s) to be Amended]Brief explanation of the drawings
[Method of Amendment]Addition
[Proposed Amendment]
[Brief Description of the Drawings]
[Drawing 1]It is a microphotograph showing the honeycomb structured body of this invention.

[Translation done.]